A highly immunogenic trivalent T cell receptor peptide vaccine for multiple sclerosis

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Background: T cell receptor (TCR) peptide vaccination is a novel approach to treating multiple sclerosis (MS). The low immunogenicity of previous vaccines has hindered the development of TCR peptide vaccination for MS. **Objective:** To compare the immunogenicity of intramuscular injections of TCR BV5S2, BV6S5 and BV13S1 CDR2 peptides in incomplete Freund's adjuvant (IFA) with intradermal injections of the same peptides without IFA. **Methods:** MS subjects were randomized to receive TCR peptides/IFA, TCR peptides/saline or IFA alone. Subjects were on study for 24 weeks. **Results:** The TCR peptides/IFA vaccine induced vigorous T cell responses in 100% of subjects completing the 24-week study (9/9) compared with only 20% (2/10) of those receiving the TCR peptides/saline vaccine (P =0.001). IFA alone induced a weak response in only one of five subjects. Aside from injection site reactions, there were no significant adverse events attributable to the treatment. **Conclusions:** The trivalent TCR peptide in IFA vaccine represents a significant improvement in immunogenicity over previous TCR peptide vaccines and warrants investigation of its ability to treat MS.
Multiple Sclerosis (2005) **11**, 552–561

Introduction

Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system (CNS). T cells specific for myelin antigens, particularly myelin basic protein (MBP), proteolipid protein and myelin oligodendrocyte glycoprotein, are believed to be central to the pernicious immune response in MS.[1–12] Since myelin-reactive T cells are present in the blood of normal controls as well as in MS patients, MS most likely involves impaired regulation of myelin-reactive T cells. In support of this hypothesis, several laboratories have found evidence of deficits in regulatory or suppressor T cells in MS patients.[13–16] In addition, previous work by Khoury et al. has correlated changes in activated T cells in blood with disease activity.[17] Finally, several lines of evidence indicate that MBP specific T cells in the blood and cerebrospinal fluid of patients are activated, suggesting a deficiency of autoregulatory mechanisms.[3,5,6,8] Development of therapies that enhance regulatory T cells capable of inhibiting myelin reactive T cells remains a major goal for the advancement of the treatment of MS.

Seminal observations by Vandenbark et al.,[18] and Howell et al.,[19] demonstrated that T cell receptor (TCR) peptides from the complementarity determining regions 2 (CDR2) or 3 (CDR3) of a disease associated beta variable (BV) gene prevented and suppressed an animal model of MS, experimental autoimmune encephalomyelitis (EAE). Subsequent studies demonstrated that vaccination with a TCR CDR2 peptide could treat EAE after disease onset, and that the mechanism of action involved boosting natural regulatory TCR reactive T cells that inhibited the activity of disease causing myelin reactive T cells.[20,21] Similar results were reported by other laboratories.[22,23] Based on the findings in EAE, TCR peptide vaccination appeared to be a promising new approach for the treatment of MS and other human T cell mediated autoimmune diseases.[24]

Development of TCR peptide vaccination as a treatment for MS required identifying target TCR BV genes and creating a vaccine capable of reliably boosting TCR reactive T cells. Research from several laboratories using different experimental approaches indicated that two related TCR families, BV5 and BV6, were commonly used by MBP specific T cells in the blood, cerebrospinal fluid (CSF) and brain of MS patients.[25–27] In addition, a meta-analysis was performed analysing 148 MBP T cell isolates from 24 patients to determine the gene usage and frequency in MS patients.[24] The results of this analysis suggested that, in addition to the predominant BV5 and BV6 gene families, BV13S1 was also over-expressed consistently, but by a lower percentage of MBP specific T cells. These findings suggested that the pathological response to MBP in >90% of MS patients in part involves T cells that utilize BV5, BV6, or BV13 genes, providing a rationale for immunizing MS patients with synthetic TCR peptides from these gene families.

In 1994, Bourdette et al. reported the results of the first clinical trial of TCR peptide vaccination in MS.[28] Their approach entailed intradermal (ID) injection of individual TCR peptides in saline and the use of a quantitative limiting dilution assay (LDA) to detect immunologic responses to the peptides. In this initial study, seven out of 11 MS patients injected with a CDR2 TCR BV5S2 peptide that contained a single amino acid substitution at position 49 (Y49T) and six of 11 patients treated with a CDR2 TCR BV6S1 peptide developed significant increases in circulating TCR peptide specific T cells. This same group of investigators subsequently reported on a double blind, placebo controlled Phase I/II trial in patients with progressive MS that demonstrated successful vaccination in 55% of subjects treated with the substituted Y49T TCR BV5S2 peptide.[29] Importantly, subjects successfully vaccinated with the BV5S2 peptide had a statistically significant decrease in circulating MBP specific T cells compared to subjects who did not respond to the vaccine or received placebo. In addition, none of the subjects (0/6) who responded immunologically to the BV5S2 peptide had worsened clinically by the end of the one-year study, whereas 59% (10/17) of those who did not have an immune response to the peptide or received placebo had worsened.[30] Additional studies indicated that the human TCR peptide specific T cells boosted by the TCR peptide vaccine produced Th2-type cytokines, including interleukin (IL)-4 and IL-10, the latter of which suppressed proliferation and interferon-γ production by MBP specific T cells.[31–33] These initial studies demonstrated that vaccination with a single BV5S2 peptide boosted regulatory Th2-type TCR reactive T cells with a resultant decrease in circulating MBP reactive T cells and possible clinical benefit. However, ID injection of the BV5S2 TCR peptide induced immune responses in no more than 50–60% of MS patients, limiting the utility of this vaccine for clinical efficacy trials.

In 1997, Gold et al. reported on the vaccination of a small number of MS subjects with a TCR BV6S5 CDR2 peptide emulsified in incomplete Freund's adjuvant (IFA) and administered by IM injection.[27,34] Although T cell frequencies were not quantified in this study, vaccination with the BV6S5 peptide/IFA appeared to induce significant proliferation responses to the TCR peptide in about 90% of patients. While there was no apparent clinical benefit in this small, short duration trial, there was evidence of a decrease in activated T cells bearing BV6S5 within the CSF of vaccinated subjects, suggesting that the vaccine was capable of inducing regulation of activated T cells within the CNS. Vaccination with different TCR peptides in IFA has also been used to boost regulatory T cells in patients with rheumatoid arthritis and psoriasis as well as MS.[24,35,36] These studies suggested that use of IFA as an adjuvant might increase the immunogenicity of TCR peptides.

The primary purpose of the current study was to compare the immunogenicity and safety of IR902, a combination of the three CDR2 TCR peptides (BV5S2, BV6S5 and BV13S1) emulsified in IFA, with that of IR903, a vaccine composed of the same peptides administered individually in saline without IFA. Our results clearly demonstrate that the IR902 trivalent TCR peptide vaccine in IFA is superior to IR903 in its ability to induce an immune response to the peptides. IR902 is capable of safely boosting TCR reactive T cells in 100% of MS patients, providing for the first time a highly reliable immunogenic TCR peptide vaccine for use in clinical trials for the treatment of MS.

Methods and patients

Study design

This was a three-arm, Phase I/II randomized, partially blinded, multi-center trial comparing the immunogenicity of three TCR CDR2 peptides from BV5S2, BV6S5 and BV13S1 when given with and without adjuvant. A third group received IFA alone. Subjects were screened and enrolled at four sites in Oregon and Washington. The study was approved by the Investigational Review Committees at each site. At the initial screening visit (Week −4), subjects signed an informed consent and then were evaluated to determine if they met inclusion/exclusion criteria. Subjects meeting criteria returned four weeks later for the Week 1 visit during which they were randomized and received their first injection. Subjects randomized to receive TCR peptides in saline (IR903) returned for injections on Weeks 2, 3, 4, 8, 16, and 20. Subjects randomized to receive TCR peptides/IFA (IF902) or IFA alone returned for injections on Weeks 4, 8, 12, 16 and 20 and did not have study visits on Weeks 2 or 3. Subjects returned for a Week 24 visit at which time they exited the study.

Subjects

Subjects needed to meet the following inclusion/exclusion criteria: age 18–60, inclusive; definite MS by Poser criteria;[37] relapsing remitting or secondary progressive course;[38] Expanded Disability Status Score (EDSS) of ≤6.5;[39] at least one clinical relapse or a brain magnetic resonance imaging (MRI) scan with at least one

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gadolinium enhancing lesion within the preceding 24 months; brain MRI consistent with diagnosis of MS; no corticosteroids or interferon-beta within 30 days of enrollment; no glatiramer acetate, azathioprine, methotrexate or cyclophosphamide within 90 days of enrollment; no previous treatment with TCR peptides, whole T cell vaccination, cladribine, cyclosporine A or mitoxantrone; no serious medical or psychiatric disorder. Because a previous multi-center trial of TCR VB5S2 peptide vaccination did not demonstrate a difference in immune responses between HLA-DR2+ and HLA-DR2− MS subjects (unpublished results), HLA-DR status was not used as an inclusion/exclusion criterion. Women of childbearing potential could not be pregnant and had to be willing to use an acceptable form of birth control. Subjects with any of the following laboratory values were excluded: creatinine $>1.5 \times$ high normal; hemoglobin <9.5 mg/dL; platelets $<75\ 000/mm^3$; proteinuria $>1+$ without evidence of urinary tract infection; SGOT/SGPT $\geq 2.5 \times$ high normal.

TCR peptides

The TCR CDR2 peptides (BV5S2, BV6S5 and BV13S1) used in this study were prepared by the Immune Response Corporation (IRC) and consisted of 21, 20 and 19 amino acids, respectively. The amino acid sequences were as follows: BV5S2: 38-Ala-Leu-Gly-Gln-Gly-Pro-Gln-Phe-Ile-Phe-Gln-<u>Thr</u>-Tyr-Glu-Glu-Glu-Glu-Arg-Gln-Arg-Gly-58 {Note: This peptide has a threonine substituted for tyrosine at position 49 (underlined) and has been previously shown to be more immunogenic than the native sequence}[29]; BV6S5: 39-Leu-Gly-Gln-Gly-Pro-Glu-Phe-Leu-Thr-Tyr-Phe-Gln-Asn-Glu-Ala-Gln-Leu-Glu-Lys-Ser-58; BV13S1: 42-Gly-Leu-Arg-Leu-Ile-His-Tyr-Ser-Val-Gly-Ala-Gly-Ile-Thr-Asp-Gln-Gly-Glu-Val-60.

Vaccine preparation and administration

Vaccine IR902 (NeuroVax TM) was prepared as a 1:1 mixture of the three peptides in aqueous solution and IFA (10% surfactant Montanide 80–90% Drakeol 6 VR light mineral oil supplied by Seppic, Inc). Each single-dose syringe contained 100 µg/mL of each peptide in a nominal volume of 1.1 ± 0.2 mL. Single-dose syringes of a 1:1 mixture of aqueous solution without peptide and IFA were prepared for the IFA controls and were indistinguishable in appearance from IR902. The adjuvant free vaccine, IR903, consisted of three single-use pre-filled syringes (one for each peptide) containing 100 µg of peptide in 0.9% saline in a nominal volume of 0.1 ± 0.02 mL. Pre-filled syringes were for single use only and were stored at 2–8°C until use. IR902 and IFA were injected into the deltoid muscle (alternating between the arms) every four weeks for the duration of the study. IR903 was injected intradermally on the volar surface of the forearm weekly (alternating between the arms) for four weeks and then every four weeks for the remainder of the study.

Limiting dilution assay (LDA) to quantify TCR reactive T cell frequencies

A LDA was used to determine the circulating frequencies of T cells specific for each of the TCR peptides, as previously described.[33] Briefly, peripheral blood mononuclear cells (PBMC) were cultured in 96-well microtiter plates at dilutions of 5, 2.5, 1.25 and 0.625×10^5 cells/well. At cell concentrations $\leq 1.25 \times 10^5$, 10^5 additional irradiated autologous PBMC were added to each well to serve as antigen presenting cells. Twelve replicate wells were cultured at each cell concentration. Cultures received 25 µg/mL of each peptide tested separately, 75 µg/mL of the combined tripeptide mixture, or culture medium (control), and tritiated thymidine uptake was measured after five days. Individual wells were scored positive if the counts per minute exceeded 1.96 standard deviations (95% CI) of the mean of wells cultured at the same cell concentration without antigen. By using the percentage of non-responding wells at each cell concentration, TCR peptide specific T cell frequencies and their 95% CI were estimated by the X^2 minimization method,[40] employing a program adapted for use with a personal computer. This method of analysis provided an estimated T cell frequency with a 95% CI, permitting statistical comparison of frequencies obtained at different times from the same subject.

Determination of response to vaccination

Subjects underwent LDA for each of the three peptides alone and for the tripeptide mixture twice before receiving injections (at Week −4 and Week 1 immediately before first injection) and at weeks 4, 8, 16 and 24. Subjects were considered to have responded immunologically to an individual TCR peptide or the tripeptide mixture if two or more LDA frequency determinations were significantly elevated above the reference baseline frequency (Week 1) and at least one of these post-immunization frequencies was $>2 \times 10^{-6}$. Subjects were considered to have been a 'responder' to the TCR peptide vaccine if they had a significant immunologic response to at least one of the three peptides or the tripeptide mixture. Subjects who did not respond to any of the three peptides or the tripeptide mixture were considered to be 'non-responders' to the vaccine. All LDA were performed blinded to treatment status.

Toxicity monitoring

At entry and Weeks 8 and 24 visits, samples were taken from subjects for a complete blood count, a 24-channel chemistry panel, and a urinalysis. Adverse events were recorded at each study visit.

Clinical measures

An EDSS, 25-foot timed walk, and 9-hole peg test were performed at the Week –4 visit and repeated at the Weeks 8, 16 and 24 visits.

Brain MRI

Subjects underwent brain MRI at Weeks 1 (pre-immunization), 16, 20, and 24 at each study site following a

standardized protocol. All scans were performed on General Electric 1.5 Tesla scanners. Pre-contrast T1-weighted spin echo (SE), proton density and T2-weighted axial images were obtained at each visit. Post-contrast axial T1-weighted SE images were acquired five to seven minutes after intravenous administration of gadolinium diethylenetriamine pentaacetate (0.1 mmol/kg). Images were stored digitally and transferred to a central site for review. A radiologist blinded to treatment status identified enhancing lesions on each scan. A scan was considered 'active' if there were one or more enhancing lesions.

Randomization and blinding

Subjects were randomized in blocks of 12 (five IR902, five IR903 and two IFA alone). Randomization was carried out using a permuted blocks design and employed no stratification. Prior to enrolling subjects, each clinical site received an initial supply of investigational product, consisting of sets of syringe kits pre-labeled with subject identification numbers. To randomize a subject, the site pharmacist assigned in numerical order a subject identification number from a pre-printed subject Assignment Log that corresponded to the pre-labeled investigational syringe kits. Subjects were randomized while physically in the clinic for the Week 1 visit. Subjects and the nurse administering injections were blinded to treatment status for those subjects receiving IR902 and IFA, but were unblinded for those receiving IR903. Physicians treating and examining the subjects, all laboratory personnel performing immunologic assays and the radiologists assessing the MRI scans were kept blinded to the treatment status of all subjects.

Sample size

The primary outcome measure of this study was the percentage of subjects who had a positive immunologic response to vaccination as determined by the LDA. The initial power analysis predicted an 18% response rate among subjects receiving IR903 and a 60% response rate among subjects receiving IR902. Given these assumptions, a sample size of 21 per treatment arm provided 80% power to detect a difference in response rates using four different statistical tests (the Mantel–Haenszel Chi-Square test, Pearson Chi-Square test, Kruskal–Wallis test and Mann–Whitney test). The study was planned to have a sample size of 25 subjects for the IR902 and IR903 arms to allow for drop-outs. Because LDA for TCR peptide responses had not been previously performed on subjects receiving IFA alone, the study design included enrollment of ten subjects into the IFA arm. Thus, the original design called for enrolling 60 subjects. However, the Sponsor performed an interim analysis in February 2002 on the blinded LDA results from the first 20 subjects, including 18 who had completed the study, and determined that there was a highly significant difference for the primary endpoint between one treatment group and the other two treatment groups. At that point, the Sponsor decided to discontinue the study early as the primary endpoint had been achieved. Thirty-seven subjects had been enrolled at the time the Sponsor discontinued the study.

Statistical analyses

Rates of immunological responses among treatment groups or between responders versus non-responders were evaluated by the Mantel–Haenszel Chi-Square test, Pearson Chi-Square test, Kruskal–Wallis test and Mann–Whitney test. Significance was established at $P \leq 0.05$. For the MRI analysis, at baseline the fraction of TCR responders with active scans was compared with that of non-responders using Fisher's exact test. To account for baseline activity, at Weeks 16, 20 and 24, the fraction of TCR responders with active scans was compared with that of non-responders using 2×2 contingency tables stratifying by baseline activity on MRI. For the stratified tables, Zelen's exact test was used to test for the homogeneity of the odds ratio. The P-values for the hypothesis of common odds ratio different from one, and exact CI for the common odds ratio were determined using Stat-Exact v. 6.02 (Cytel Corporation, 2004). The number of gadolinium-enhancing lesions was compared between the responders and non-responders at baseline, week 16, 20 and 24 using the exact version of the Wilcoxon two-sided two sample test using SAS v.8.1.

Results

Subjects

The disposition of subjects participating in the trial is summarized in Figure 1. Sixty subjects were screened and, of these, 23 did not meet inclusion/exclusion criteria, declined participation prior to randomization, or were in the screening stage when the study was discontinued. Of the remaining 37 subjects, all completed the Week 1 visit, were randomized, and received their first injection. There were 16 subjects randomized to the IR902 group, 15 to the IR903 group, and six to the IFA group. There were no significant demographic or clinical differences among the three groups at entry (Table 1).

Of the 16 subjects randomized to the IR902 group, nine completed the study and seven discontinued prior to completion (one due to worsening of MS as a result of a relapse that began before the first injection and six due to the Sponsor stopping the study early). In the IR903 group, ten subjects completed the study and five discontinued prior to completion (two due to worsening of MS and three due to the Sponsor stopping the study). In the IFA group, five subjects completed the study and one discontinued due to the Sponsor ending the study. There were thus 24 subjects who completed the 24-week study.

Frequency of responders to TCR vaccination

Using an intent-to-treat analysis, 15/16 (94%) of subjects receiving at least one injection of IR902 'responded' immunologically to the vaccine compared with 2/15 (14%) in the IR903 group and 1/6 in the IFA group ($P < 0.001$, Table 2). The one subject in the IR902 group classified as a 'non-responder' had one strongly positive post-immunization LDA (0.12 tripeptide-reactive T cells/million PBMC at baseline increased to 15.9 cells/million at Week 4) performed before discontinuation of the study,
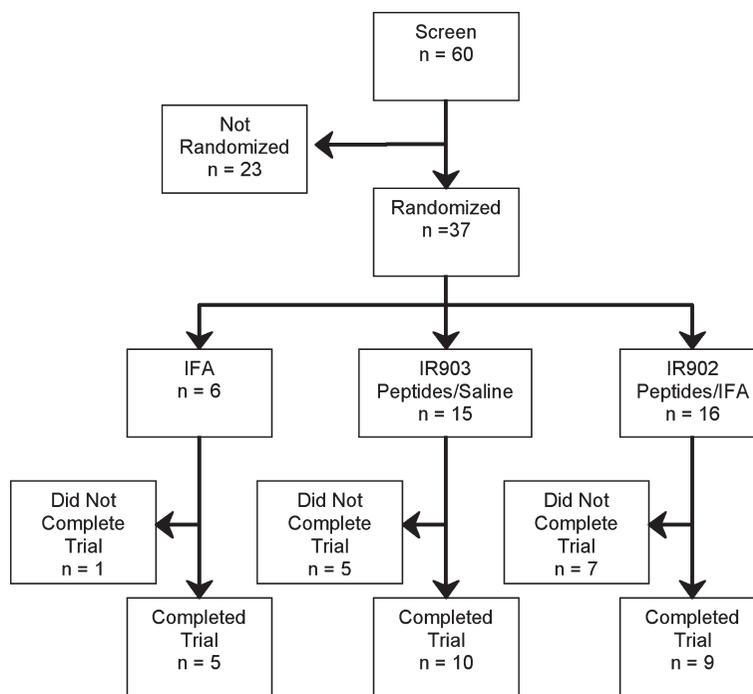
Figure 1 *Flow chart showing disposition of study subjects. For the trial, 60 subjects were screened and 37 were randomized into treatment groups, including 16 that received IR902 (tripeptides in IFA), 15 that received IR903 (tripeptides in saline), and six that received IFA alone.*

but without a second LDA evaluation, the subject could not meet the pre-determined criteria for being classified as a 'responder'. Among the 24 subjects who completed the study, 9/9 (100%) in the IR902 group responded to the vaccine compared with 2/10 (20%) in the IR903 group and 1/5 (20%) in the IFA group ($P < 0.001$) (Table 2).

Frequencies of TCR reactive T cells
Figure 2 shows the mean TCR reactive T cell frequencies over time for the three groups for each of the individual peptides and the tripeptide mixture. The LDA frequencies prior to vaccination in all three treatment groups were < 1 cell/million PBMC for all three TCR peptides and the tripeptide mixture. By the Week 8 visit, the IR902 group had a significant rise in mean frequencies for all three peptides and the tripeptide mixture compared with the IR903 and IFA groups. The highest frequencies occurred on Weeks 4 and 16 post-vaccination, but responses remained significantly elevated at the Week 24 visit.

Figure 3 shows the pre-vaccination and maximum post-vaccination frequencies of TCR peptide reactive T cells for the responders versus non-responders who completed the 24-week protocol. The three individual TCR peptides appeared to be comparable as immunogens (Table 3). Among the 12 subjects that completed the 24-week study and who were classified as 'responders', each peptide induced a significant immune response in a similar fraction of the responders (8/12, 8/12 and 9/12 for BV5S2, BV6S5 and BV13S1, respectively). The strength of the immune response induced was also similar, as assessed by the mean maximum peptide specific T cell frequency (17.3, 20.8 and 17.6 cells per million PBMC for BV5S2, BV6S5 and BV13S1, respectively). Among the IR902 group that received at least one immunization, 13/15 of the responders had significant immune responses to all three peptides. In the IR903 group, one of the two responders had weakly positive LDAs only to the BV5S2 peptide and the tripeptide mixture, whereas the other had

Table 1 Baseline demographics

	IR902 (n = 16)	*IR903* (n = 15)	*IFA* (n = 6)	*All* (n = 37)
Female (%)	63	60	67	62
Age (years) (mean ± SD)	45 ± 8	44 ± 9	44 ± 7	45 ± 8
RRMS:SPMS	12:4	10:5	4:2	26:11
RRMS (%)	75	66.7	66.7	70
SPMS (%)	25	33.3	33.3	30
MS duration from onset (years) (mean ± SD)	11 ± 8.3	14 ± 8.6	11 ± 8.6	12 ± 8.3
EDSS at screen (mean ± SD)	3.8 ± 1.9	4.1 ± 2.4	3.7 ± 2.0	3.9 ± 2.0
Gadolinium + lesions (mean (range))[a]	1 (0−4)	0.6 (0−2)	0.2 (0−1)	0.7 (0−4)
Percent of subjects with active scans[a]	44	40	20	38

[a]MRI data are for subset of 24 subjects who completed the trial.

Table 2 Fraction of subjects who were TCR peptide responders by treatment arm

Treatment arm	Responders/ randomized subjects (n = 37)	Responders/ subjects completing study (n = 24)
IFA	1/6	1/5
IR903 (peptide/saline)	2/15	2/10
IR902 (peptide/IFA)	15/16*	9/9†

*$P < 0.001$.
†$P < 0.001$.

weakly positive LDAs only to the BV13S1 peptide and the tripeptide mixture. The single responder in the IFA group had weakly positive LDAs only to the BV13S1 peptide and the tripeptide mixture (maximum frequency of 3.2 cells/ million PBMC).

LDA response to tripeptide mixture represents the sum of responses to individual TCR peptides

Evaluation of individual LDA response patterns for each patient indicated that the T cell frequency measured in response to the tripeptide mixture approximated the sum of the T cell frequencies to the individual TCR peptides. A regression analysis shown in Figure 4 demonstrated
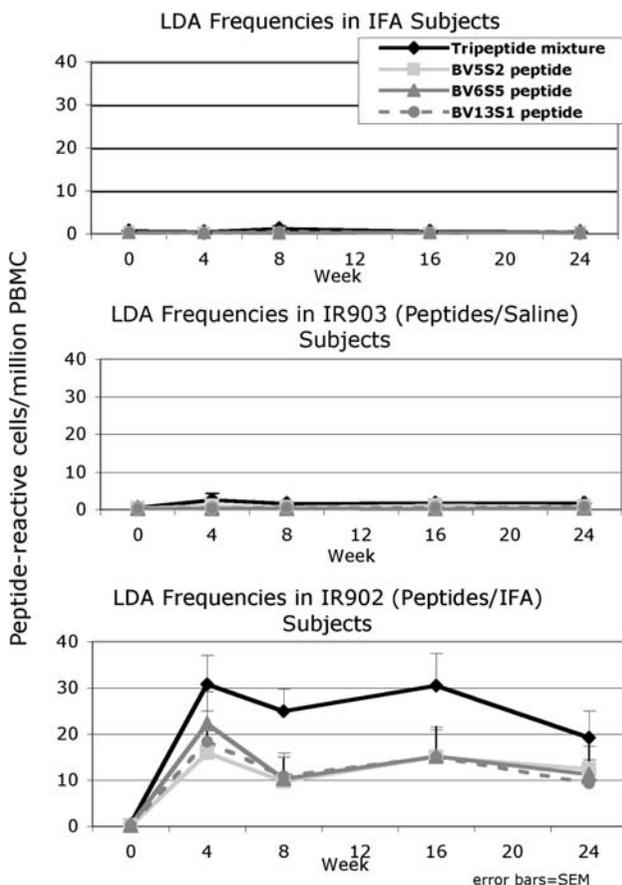

Figure 2 *Mean LDA T cell frequencies to the tripeptide mixture and individual peptides among subjects in three treatment arms who completed the 24 week trial.*

nearly a 1:1 correlation (slope of 0.96) and a highly significant correlation (R^2 value of 0.91, $P < 0.001$). A similar result was obtained when including or excluding summed individual frequencies or tripeptide responses that exceeded the maximum detectable frequency of the LDA assay (plotted as the maximum of 47.7 cells/million PBMC). These data indicate that distinct populations of T cells were stimulated by each individual TCR peptide and that responses to multiple peptides in the vaccine potentiated activity in an additive manner.

Adverse events

There were four serious adverse events reported during the study and the site principal investigators considered none of these related to the study medication. One subject in the IFA group was hospitalized for management of intractable neurogenic pain secondary to MS. One subject receiving IR902 was hospitalized for ileal loop surgery to treat chronic urinary incontinence. One subject receiving IR903 was hospitalized twice for treatment of a severe relapse of MS. This subject had SPMS, had a relapse that commenced before the first injection of IR903 and did not develop an immune response to the IR903.

The only adverse events reported during the study that were considered related to study drug were injection site reactions, including erythema, induration, itching, pain and discoloration. Injection site reactions occurred in all three groups. All were mild, and transient in nature. Subjects in the IR903 group experienced injection site reactions most commonly.

Three subjects exited the study because of worsening of their MS. One subject receiving IR902 had a relapse of MS that commenced shortly before receiving the first dose of IR902 and discontinued the trial because of continued worsening after the first injection. This subject agreed to return for scheduled visits for LDA assays, allowing determination of the immune response to the single injection of IR902. This subject had a strong immunologic response to IR902 that persisted throughout the study. Two subjects discontinued IR903 because of worsening of MS, including the subject hospitalized twice for a severe relapse of MS. Neither of these subjects developed an immune response to the IR903 vaccine.

Changes in clinical measures

As expected for this short-term immunogenicity study, there were no significant differences among the three treatment groups in changes in EDSS, 25-foot timed walk, or 9-hole peg test over the course of the study. When subjects were analysed in two groups, TCR peptide responders versus non-responders, there were no significant differences in changes in any of the clinical measures.

MRI

Disease activity on brain MRI was assessed by fraction of subjects with active scans (based on the presence of at least one gadolinium enhancing lesion) and average number of gadolinium enhancing lesions. The MRI-based disease activity of TCR peptide responders versus non-responders was compared at baseline and Weeks 16, 20
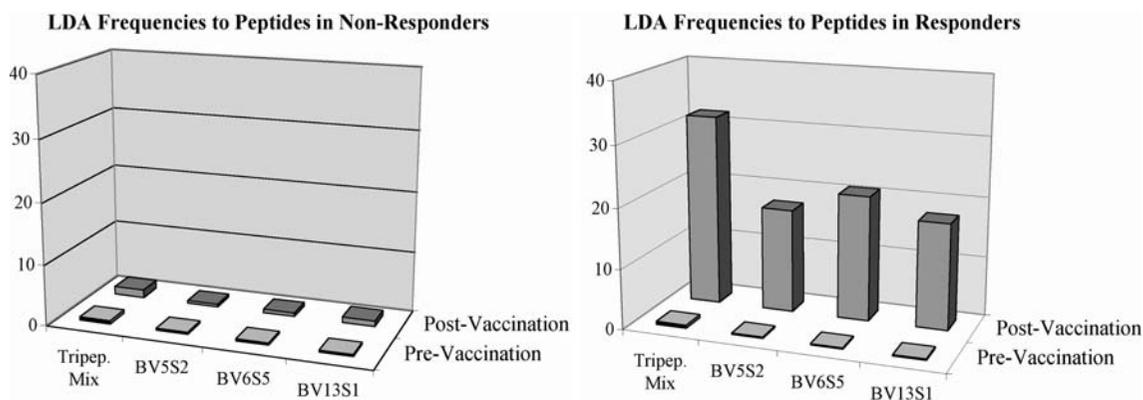
LDA Frequencies to Peptides in Non-Responders

LDA Frequencies to Peptides in Responders

Figure 3 Mean LDA T cell frequencies to the tripeptide mixture and the individual TCR peptides pre- and post-vaccination among non-responders (n = 12) and responders (n = 12) who completed the 24-week trial. TCR responders included all subjects (nine received IR902, two received IR903 and one received IFA) that met criteria of having an immunologic response to the TCR peptides.

and 24. As shown in Figure 5(A), 42% of TCR responders and 33% of TCR non-responders had active MRI scans at baseline ($P = 1.0$). After adjusting for differences between the two groups at baseline, there were no statistically significant differences between the two groups in fraction of subjects with active scans at Weeks 16, 20 and 24 (P values of 0.67, 1.0 and 0.16, respectively). However there was a statistical trend at Week 24 favoring a reduction in activity in the TCR responder group (25% of TCR peptide responders had active scans versus 55% for TCR peptide non-responders, $P = 0.16$). A similar trend was noted for the mean number of gadolinium positive lesions. TCR peptide responders at baseline had a mean of 0.42 gadolinium positive lesions compared with a mean of 0.33 for the TCR peptide non-responders ($P = 0.47$). There were no statistically significant differences between the mean number of gadolinium positive lesions between the two groups at Weeks 16, 20 and 24 (P values of 0.50, 0.70 and 0.27, respectively). A trend was again seen at Week 24 suggesting a decrease in mean number gadolinium enhancing lesions in the TCR responders compared with the TCR non-responders (mean of 0.25 for TCR peptide responders versus 0.54 for TCR peptide non-responders, $P = 0.27$). Thus, by both MRI parameters tested, the TCR peptide responder group tended to have less disease activity by Week 24, although the differences between responders and non-responders did not achieve statistical significance.

Table 3 Frequency of T cells specific for individual TCR peptides among TCR peptide responders ($n = 12$)

	BV5S2*	BV6S5*	BV13S1*
Maximum post-immunization frequency (mean ± SEM cells/10^6 PBMC)	17.3 ± 5.6	20.8 ± 5.8	17.6 ± 6.0
Range (cells/10^6 PBMC)	6.4–47.7	7.9–47.7	2.6–47.7

*Among the 12 subjects classified as TCR peptide responders, 8/12 had a significant immune response to BV5S2, 8/12 responded to BV6S5 and 9/12 responded to BV13S1. Frequency data presented are for those individuals who responded to the given peptide.

Discussion

The data presented here demonstrate the markedly enhanced immunogenicity of the TCR tripeptide vaccine administered in IFA for inducing TCR-specific T cells. The potent activity of this formulation represents an important milestone in the development of a TCR vaccine for the treatment of MS patients. While our previous double blind, placebo controlled pilot trial suggested clinical benefit in those patients who had strong immunological responses to the vaccinating BV5S2 peptide,[29,30] the rate of response to this peptide without adjuvant ranged from only 20 to about 60% of vaccinated MS patients, depending on the trial.[24] A low rate of successful vaccination with TCR peptides in saline (20%) was again evident in the current trial. In contrast, the trivalent TCR peptide
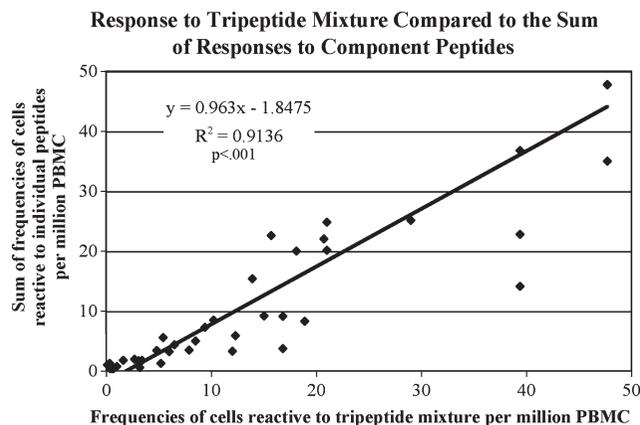
Response to Tripeptide Mixture Compared to the Sum of Responses to Component Peptides

$y = 0.963x - 1.8475$
$R^2 = 0.9136$
$p < .001$

Figure 4 Regression analysis comparing the LDA frequency of TCR-reactive T cells to the tripeptide mixture versus the sum of the frequencies to each individual TCR peptide. Values represent 53 post-vaccination comparisons from the 12 responder patients who completed the study. TCR responders included all subjects (nine received IR902, two received IR903 and one received IFA) that met criteria of having an immunologic response to the TCR peptides. Values of 47.7 cells/million for response to the tripeptide mixture represented the maximum frequencies detectable by the LDA. Sums of frequencies that exceeded this value were also plotted as 47.7 cells/million.
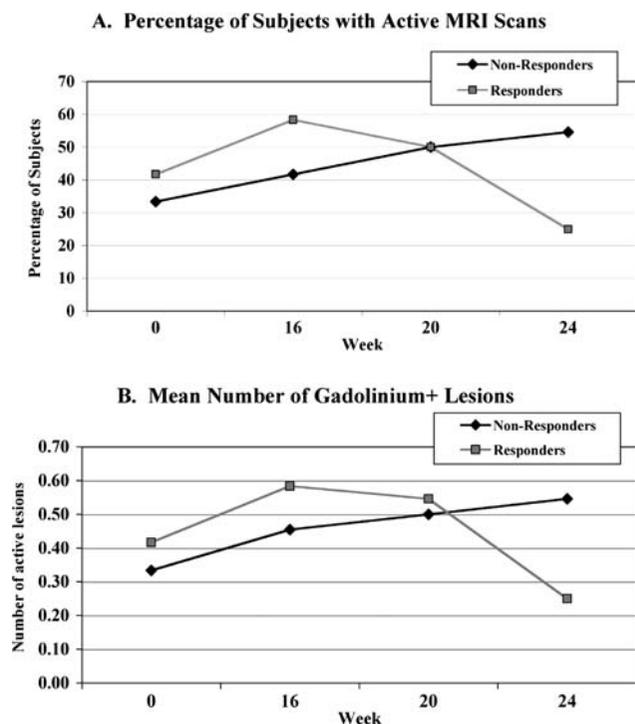
A. Percentage of Subjects with Active MRI Scans

B. Mean Number of Gadolinium+ Lesions

Figure 5 *Changes in MRI during the 24-week study. (A) Percentage of subjects with active MRI scans at baseline and weeks 16, 20 and 24 among responders* (n = 12) *and non-responders* (n = 12). *Active MRI scans had at least one gadolinium positive lesion. TCR responders included all subjects (nine received IR902, two received IR903 and one received IFA) that met criteria of having an immunologic response to the TCR peptides. (B) Mean number of gadolinium positive lesions in responders versus non-responders.*

vaccine in IFA strongly boosted circulating frequencies of TCR-reactive T cells in 100% of MS patients, with average frequencies to each peptide of 15–20 cells/million PBMC. Moreover, the responses to each of the three peptides in the vaccine appeared to be additive, further enhancing total TCR reactive frequencies in each patient. Although most of the patients in this study had RRMS, the few with SPMS that received IR902 had strong immune responses to the vaccine, indicating that this vaccine will be immunogenic in both forms of MS.

The response to the tripeptides in IFA was much more vigorous than that observed to the TCR peptides in saline that produced positive responses in the range of only 2–8 cells/million PBMC, with only occasional responses >8 cells/million. Boosting the frequencies of TCR peptide-reactive T cells was peptide dependent, since comparable changes were not observed in subjects receiving IFA alone. The lone weak responder to BV13S1 peptide in the IFA group may represent adjuvant enhancement of disease-associated perturbations of TCR network regulation. It is noteworthy that IFA alone can protect against EAE in certain mouse strains.[41]

The proposed mechanism of action of TCR peptide vaccination relates to its ability to boost a natural immunoregulatory network composed of TCR reactive T cells.[21,42] In humans, these regulatory T cells recognize processed TCR peptides presented by MHC class II molecules on the surface of activated Th1 cells.[30] Following recognition of the relevant TCR peptide, the TCR reactive T cells produce Th2-type immunomodulatory cytokines, including IL-10 that can directly downregulate the activated Th1 cells utilizing the appropriate TCR BV gene.[29,32] Release of immunomodulatory cytokines by the TCR reactive T cells could also downregulate other activated myelin reactive Th1 cells within the CNS that utilize different BV genes, a mechanism referred to as 'bystander suppression'. 'Bystander suppression' provides a mechanism through which immunization with TCR peptides selected based on TCR V gene usage of MBP specific T cells could regulate T cells specific for other myelin antigens. Moreover, cell fractions enriched in CD4 + CD25 + T cells with TCR reactivity demonstrated enhanced cell–cell contact dependent inhibition of anti-CD3 activated indicator cells in a manner characteristic of Treg cells,[14] again implicating a bystander suppression mechanism. We recently demonstrated that MS subjects compared to healthy controls have a reduction in CD4 + CD25 + Treg activity *in vitro* and have a reduction in mRNA and protein expression of the FOXP3 gene, which is associated with CD4 + CD25 + Treg cells.[43] We now have evidence that immunization with the TCR tripeptide/IFA vaccine can boost CD4 + CD25 + Treg activity and expression of FOXP3 (unpublished data). Boosting of CD4 + CD25 + Treg cells by TCR peptide vaccination could regulate an array of myelin reactive Th1 cells both in the periphery and within the CNS. Further studies are in progress investigating the ability of IR902 to boost the activity of CD4 + CD25 + Treg cells and expression of FOXP3 in MS subjects.

The current study represents the seventh trial using various TCR peptides to induce TCR reactive T cells involving 195 MS subjects.[24] All previous trials utilized a single BV CDR2 peptide, in most cases BV5S2 or BV6S5, which were both utilized in the present study. The third peptide used in this study, BV13S1, was used previously to treat patients with psoriasis,[24] but had not previously been tested in MS patients. This is the first clinical trial of TCR peptide vaccination in MS utilizing multiple TCR peptides. These three peptides were chosen for the IR902 vaccine because of evidence that >90% of MS patients utilized TCR BV5, BV6 or BV13 genes in at least some MBP specific T cells, thus suggesting that these BV genes are disease relevant.[24] The IR902 vaccine induced strong immune responses to all three TCR peptides with most subjects (13/15 responders) developing an immune response to all three peptides. Only two of the subjects who immunologically responded to IR902 failed to respond to all three peptides, although they did respond to two of the three peptides in the vaccine. Thus, IR902 is capable of boosting regulatory TCR reactive T cells directed at three BV genes that are over-utilized by MBP-specific T cells from most MS patients.

This is the first study in which MRI changes were assessed prior to and after TCR peptide vaccination. Given the small sample size and the relatively short duration of the study, it is not surprising that no statistically

significant differences were found in MRI activity between the TCR responder and non-responder groups. There was a trend towards decreased disease activity, as measured by the fraction of subjects with active scans and the mean number of gadolinium enhancing lesions 24 weeks after initiating vaccination in the TCR responders compared with the TCR non-responders. This trend might simply reflect natural fluctuation of disease activity. However, it is also possible that TCR peptide vaccination may induce a delayed change in disease activity as detected by MRI. These preliminary results suggest that TCR peptide vaccination does not rapidly decrease MRI disease activity, as occurs with recombinant interferon beta and natalizumab,[44,45] but may have a delayed effect, as occurs with glatiramer acetate.[46] These initial MRI results will be useful for planning future studies of TCR peptide vaccination using MRI as an outcome measure.

In summary, our results demonstrate that the trivalent TCR peptide/IFA vaccine IR902 reliably induced vigorous T cell responses to each of the component peptides, was safe and well-tolerated, did not cause clinical worsening and showed a trend towards a delayed reduction in brain MRI activity. We are presently assessing whether less frequent injections of IR902 are as immunogenic as monthly injections and also extending our investigations on the mechanism of action of TCR peptide vaccination. Given its safety profile and consistent ability to induce TCR reactive T cells, the trivalent TCR peptide/IFA vaccine warrants a larger and longer duration clinical efficacy trial in MS.

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